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ORIGINAL PAPER

Effects of methanol-activated biochar on tetracycline concentration and soil microbial activities in the presence of copper

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Abstract

Nowadays, antibiotic-polluted soils have raised concerns regarding the health of soil ecosystems. It has been suggested that biochar can be used as an amendment to improve soil health conditions in polluted sites. The present research aimed to investigate the influence of walnut shell biochar and methanol-activated walnut shell biochar on tetracycline concentration in the presence of copper and the assessment of soil biological parameters (microbial biomass, urease and dehydrogenase enzymes). Therefore, a factorial experiment was conducted as a completely randomized design. The factors included two biochar levels without or with copper (0% biochar (control soil), soil amended with 5% walnut shell biochar and soil amended with 5% methanol-activated walnut shell biochar) and two tetracycline levels (0 and 400 mg/kg soil), which were measured at 10-, 30- and 50-day intervals. The results revealed that biochar and methanol-activated biochar efficiently decreased tetracycline concentration up to 46.5% and 80.4%, respectively. The application of biochar decreased tetracycline concentration through the formation of a stable complex with tetracycline and the reduction of its extractability as well as biodegradation. The decrease in tetracycline extractability was also facilitated by the presence of copper. Neither biochar had significant effects ($P > 0.05$) on microbial biomass. They also reduced enzymatic activities, probably due to a high application rate. However, they weakened the negative effects of tetracycline on microbial activities. It was concluded that the application of biochar in tetracycline-polluted soils could help expedite the decrease of residual tetracycline concentration, which, in turn, could improve soil biological parameters and soil health.

Keywords Dehydrogenase activity · Microbial biomass · Organic pollutant · Safe management · Soil amendment · Urease activity

Abbreviations

BC	Biochar
WBC	Walnut shell biochar
WBCM	Methanol-activated walnut shell biochar
TC	Tetracycline
Cu	Copper
Cu-EDTA	Copper concentration
CEC	Cation-exchange capacity
EC	Electrical conductivity
dw	Dry weight
OM	Organic matter
AP	Available phosphorus
AK	Available potassium
TN	Total nitrogen
O	Oxygen content
N	Nitrogen content
C	Carbon content
H	Hydrogen content
K	Unamended soil

Introduction

Nowadays, pharmaceutical antibiotics have been identified as effective agents for the prevention and treatment of infectious diseases (Jang et al. 2018). Among them, tetracycline (TC) ranks second in terms of production and consumption worldwide due to its low cost (Jang et al. 2018), extensive activity against infections (Daghrir and Drogui 2013), and treatment of animal diseases (Gu and Karthikeyan 2005; Zhang et al. 2011).

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As a broad-spectrum antibiotic, the excessive use of TC has increased the possibility of its occurrence in various environments (Luo et al. 2011; Ngigi et al. 2020a, b). Organic fertilizers (Chessa et al. 2015) and wastewater irrigation (Pan and Chu 2017) are considered two common sources that increase the concentration of TC in soil. It has been reported that TC can affect the sustainability of soil ecosystems by inhibiting bacterial activities, disrupting microbial metabolisms (Liu et al. 2020), reducing enzymatic activities (Thiele-Bruhn and Beck 2005), and developing antibiotic resistance genes (Duan et al. 2017). The development of antibiotic resistance genes in soil, especially agricultural soils, not only has a negative impact on the soil microbial community, but is also considered a potential risk to human health (Liu et al. 2020). Therefore, understanding TC behavior in soil is of particular importance to provide effective measures to reduce the negative impact of this bioactive pollutant on the soil community.

Based on the findings of previous studies, biochar (BC) is a carbonous product of the pyrolysis process (Li et al. 2019) which can be used as an amendment in sustainable soil management. BC can improve soil quality and soil health because it can provide nutrients, increase soil fertility (Choudhary et al. 2021), improve water holding capacity (Verheijen et al. 2019), increase cation-exchange capacity (CEC) and soil pH (Hailegnaw et al. 2019). It has been proved that these BC effects depend on the physical and chemical properties of BC, which are mostly affected by the raw material and pyrolysis temperature (Mitchell et al. 2015; Beheshti et al. 2018). It has also been reported that BC has unique properties such as specific surface area, surface charge, porosity, surface functional groups and aromatic structures (Ahmad et al. 2014). These properties can affect the environmental behavior of various organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Li et al. 2019), herbicides (Li et al. 2018), antibiotics (Chen et al. 2018a, b) and heavy metals (Meier et al. 2017; Moore et al. 2018) in a variety of environments. Therefore, it seems that the application of BC as a cost-effective and eco-friendly technique can affect the chemical and biological behavior of TC in soil and help to improve soil health. To date, only a few number of studies have been examined the effects of BC on the environmental behavior of TC in soil and the reduction of its negative effects on soil microbial community (Yue et al 2019; Liu et al. 2020). More studies are required to better understand the fate and behavior of TC in amended soil with BC. Moreover, BC activation or modification is a common technique to ensure the effect of BC on TC at high concentrations (Jing et al. 2014). The activating agent is usually acidic (Chen et al. 2018a, b) and alkaline (Luo et al. 2018) solutions with different concentrations. Based on the literature, this technique is mostly used to remove tetracycline from the solution medium. The effects of the application of activated BC in TC-contaminated soil are still unknown.

On the other hand, heavy metals exist with different concentrations due to human activities and lithogenic sources in soil (Pan et al. 2016; Wang et al. 2008). Among heavy metals, the use of copper (Cu) as a growth parameter in animal feed has led to increasing in Cu concentration in livestock manure (Wang et al. 2020). The application of manure in soil has increased the possibility of the simultaneous presence of TC and Cu in the environment (Wang et al. 2008). Previous studies have shown the effect of heavy metals on the change of TC chemical behavior in solution medium (Zhao et al. 2013; Kong et al. 2014). In the soil solution phase, different compounds affect each other's behavior. Therefore, considering the presence of Cu in soil environment on the behavioral changes of TC can help to better understand the fate of TC in soil. Finally, microorganisms play a central role in many ecosystem processes such as decomposition and nutrient cycling (Bloem and Breure 2003). Some soil microorganisms show a higher sensitivity to environmental disturbances (Anderson 2003). Antibiotics are also designed to affect microorganisms. Hence, the ecotoxicological effects of antibiotics can affect the natural soil microbial community even at low concentration rates (Liu et al. 2009). Previous studies have also demonstrated that high concentrations of antibiotics can alter the original functions of microorganisms such as enzymatic activities (Martinez 2008). Soil enzymes play a fundamental role because they participate in ecosystem process such as biochemical transformations, biodegradation, and biogeochemical cycles of nutrients (Oleszczuk et al. 2014). Soil enzymes are also very sensitive indicators so any environmental changes affect their performance. Therefore, their proper function can be considered as an indicator of soil health (Bera et al. 2016). The negative effects of TC on urease, dehydrogenase, and phosphatase activities have mostly been demonstrated in different studies (Liu et al. 2015; Molaei et al. 2017). The activity of these enzymes is often used for monitoring impacts of pollutants on soil health (Oleszczuk et al. 2014).

Objectives of this study were (1) to study the effect of walnut shell BC (WBC) and methanol-activated WBC (WBCM) on the extractability of residual TC concentration, (2) to investigate the effect of the presence of Cu on the extractability of residual TC concentration and (3) to study the simultaneous Cu and BC presence on the biological effects of TC (microbial biomass, urease and dehydrogenase activity) in soil during a 50-day incubation experiment. The present study was carried out during the period of December 2018 and June 2019. All experiments were performed at the Soil Science Lab, located on campus of Ferdowsi University of Mashhad, Iran.

Materials and methods

Chemicals

Tetracycline hydrochloride ($C_{22}H_{25}ClN_2O_8$) with high purity was purchased from Sigma-Aldrich Company. The acetonitrile, methanol, formic acid (HPLC grade) and all the other chemicals used in this study were obtained from Merck Chemical Company. Deionized water and distilled water were used during all the experimental procedures.

Preparation of walnut shell biochar and methanol-activated walnut shell biochar

Walnut shells were collected and washed using distilled water, and they were dried in an oven at 90 °C for 12 h. They were then powdered and were sieved with a 0.5-mm steel sieve. The prepared feedstock was pyrolyzed at a 600 °C temperature for 2 h under the oxygen-limited condition (Mitchell et al. 2015). For activation, WBC and methanol were mixed at a rate of 1:10 (V/W) and were shaken at 180 rpm for 24 h. Samples were then dried at 90 °C for 6 h in an oven (Jing et al. 2014). WBC and WBCM properties are illustrated in Table 1. Elemental composition was measured with an Elemental Analyzer (Thermo Finnigan FLASH EA 1112 Series). The O content was calculated by mass difference. The WBC yield was determined by weighing feedstock before and after pyrolysis. Ash parameter was measured by the remaining mass after heating at 750 °C for 4 h in a furnace. The electrical conductivity (EC) and the pH were measured in the solid–liquid ratio of 1:20 (Li et al. 2016a, b). The functional groups of WBC were determined by FTIR spectrometer (Thermo Nicolet AVATAR 370 FT-IR) in the range of 4000–400 cm^{-1} before and after activation (Fig. 1).

Sampling and soil analysis

A soil composite sample was collected by an auger from the top layer (0–30 cm) of an agricultural field, located in Mashhad in Khorasan Razavi Province, northeastern Iran. The soil sample was then transferred to the laboratory, air-dried and stored dry in plastic bags. Physical and chemical soil characteristics are exhibited in Table 2. Both the soil pH and the EC were determined using the saturated paste extraction method (Richards 1954). The soil texture was measured by the hydrometer method (Gee and Bauder 1986). The organic matter carbon (OM) was determined by Walkley and Black's method (1934). The total nitrogen (TN) was determined by Kjeldals' method (Bremner and Mulvaney 1982). Moreover, available phosphorus (AP) and available potassium (AK) were extracted by the sodium bicarbonate (Olsen 1954) (analyzed by the colorimetric method) and ammonium acetate (Chapman 1965) (analyzed by the flame photometer detection). The cation exchange capacity (CEC) was measured by the ammonium acetate extraction (Chapman 1965). The Cu was extracted by diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) and analyzed by the atomic absorption spectroscopy (PG-990) (Lindsay and Norvell 1978).

Experimental design

The laboratory experiments were performed at the Soil Science Lab at Ferdowsi University of Mashhad, Iran. In order to prepare different treatments, the Cu-spiked soil was prepared by spiking Cu solution ($CuSO_4.5H_2O$) (Aponte et al. 2020). Briefly, 14 kg of dried soil was weighed, spiked with Cu solution and mixed thoroughly. The equivalent Cu amount was 25 mg kg^{-1} in the dry soil. The Cu-spiked and non-spiked samples were daily hydrated with deionized water to be maintained at 70% of the water-holding capacity. Both samples were kept in plastic bags and were thoroughly mixed every day for thirty days. After the equilibration time (30 days), the samples were again dried and sieved using a 2-mm steel sieve. The DTPA-Cu concentration was determined in the Cu-spiked soil sample (15.03 mg kg^{-1}). The WBC and WBCM were added to the Cu-spiked and non-spiked soils (5% w/w) and mixed thoroughly. Six soil treatments included: (1) the control soil without the Cu and the BC (K); (2) the control soil with the Cu and without the BC (K + Cu); (3) the soil with the WBC (WBC); (4) the soil with the WBC and the Cu (WBC + Cu); (5) the soil with the WBCM (WBCM); and (6) the soil with WBCM and the Cu (WBCM + Cu). The TC was dissolved in the deionized water and was sprayed into a portion of soil treatments as described above. The equivalent TC amount was 400 mg kg^{-1} in dry soil. The TC-polluted and unpolluted samples were kept in plastic containers and were daily

Table 1 Chemical properties of walnut shell biochar (WBC) and methanol-activated walnut shell biochar (WBCM)

	C (%)	H	N	O	Yield	Ash	pH (1:10)	EC (dS m^{-1})
WBC	84.8 ± 1.11	1.9 ± 0.16	0.7 ± 0.17	3.6 ± 0.16	31 ± 0.81	9.2 ± 0.21	8.9 ± 0.08	4.5 ± 0.17
WBCM	86.3 ± 0.47	1.9 ± 0.09	0.5 ± 0.15	3.9 ± 0.45	31 ± 0.81	7.4 ± 0.03	8.9 ± 0.18	4.5 ± 0.07

All values are mean ± SD (*n* = 3)

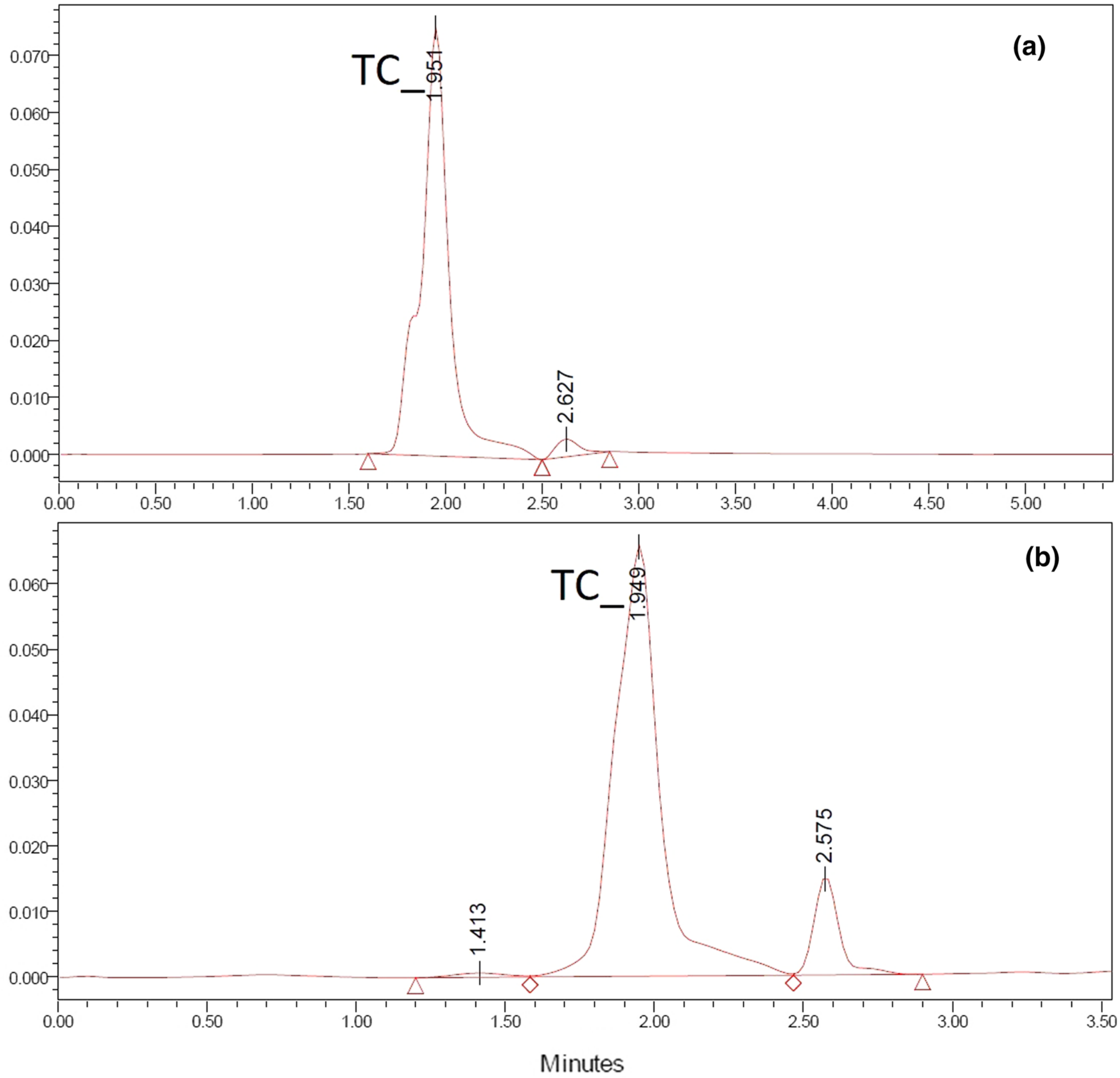

Fig. 1 High-performance liquid chromatography (HPLC) chromatograms of tetracycline **a** 100 mg L^{-1} (p.p.m) standard tetracycline **b** 100 p.p.m standard tetracycline spiked into the soil

hydrated with deionized water to be maintained at 70% of the water-hold capacity in darkness and at 25 °C temperature. After the incubation times (10/30/50 days), the samples were collected and divided into three portions. Two portions of samples were kept at 4 °C and − 20 °C for biological assessment and the determination of TC concentration, respectively. In addition, a portion of samples was dried to investigate DTPA-Cu concentration.

Laboratory analyses

Extraction of residual tetracycline

The TC was extracted by the modified QuEChERS method (Guo et al. 2016). The soil samples were extracted using the EDTA-McIlvaine buffer and were cleaned up by the d-SPE method. After evaporating by the nitrogen flow, 0.2 mL of the acetonitrile and 0.8 mL of the 0.1% formic acid were added to the samples and they were filtered by the 0.22 µm filters.

The samples were injected into the HPLC (Agilent 1260, Infinity II) which was equipped with the C18-phenomenex column (100 mm × 4.6 mm, 3 µm). The temperature was maintained at 31 °C, and the detection wavelength appeared at 254 nm. The mobile phase consisted of the formic acid (0.1%) and the acetonitrile (60:40, v/v). For the determination of TC recovery, the TC with 100 mg kg^{-1} concentration was spiked to the unpolluted soil. The TC recovery was 91% (Fig. 1).

Table 2 Physiochemical properties of the study soil

Parameters	Soil sample	Unit
Total nitrogen (TN)	505.1 ± 0.2	mg kg^{-1} dw
Available potassium (AK)	79.6 ± 0.4	mg kg^{-1} dw
Available phosphorus (AP)	5.1 ± 0.1	mg kg^{-1} dw
Copper concentration (Cu-EDTA)	0.6 ± 0.3	mg kg^{-1} dw
Electrical conductivity (EC)	1.6 ± 0.7	dS m^{-1}
Cation-exchange capacity (CEC)	6.8 ± 0.4	cmol kg^{-1}
pH	7.8 ± 0.3	–
Organic matter (OM)	0.3 ± 0.2	%
Clay	22 ± 0.2	%
Silt	34 ± 0.1	%
Sand	44 ± 0.4	%
Soil texture	Loam	–

All values are mean ± SD ($n = 3$)

Microbial and enzymatic activities

The microbial biomass was determined by the chloroform fumigation method (Jenkinson and Ladd 1981). After the titration with the $BaCl_2$, the difference between the CO_2 produced in the fumigated and unfumigated soil was calculated. The microbial biomass was reported as (μg C kg^{-1} dry soil 24 h^{-1}). The urease activity was measured by Tabatabai and Bremner's method (Tabatabai and Bremner 1972). To be concise, 5 g of the moist soil was mixed with the Toluene, the Tris Buffer (pH = 7) and the urea solution. After 2 h of incubation at 37 °C, the KCl-Ag_2SO_4 solution and the pure water were added to the samples. The released $NH4^+$-N was measured by the steam distillation apparatus and was reported as (μg NH_4^+-N g^{-1}. d^{-1}). To determine the dehydrogenase activity according to the colorimetric method (Thalmann 1968), 5 g of soil was mixed with 2,3,5-triphenyltetrazolium chloride (0.6% was the optimal concentration based on the soil texture). After 16 h of incubation at 25 °C, the acetone was added and the samples were shaken. The amount of the TPF was measured at a wavelength of 546 nm and was reported as (μg TPF g^{-1} d^{-1}).

Statistical analysis

Tetracycline concentration was statistically processed by the one-way analysis of variance (ANOVA) as a completely randomized design (CRD) in three replications. The factor included two biochar levels without or with copper (0% biochar (control), 0% biochar with copper, soil amended with 5% walnut shell biochar, 5% walnut shell biochar, 5% walnut shell biochar with copper, 5% methanol-activated walnut shell biochar, 5% methanol-activated walnut shell biochar with copper), which were measured at 10-, 30- and 50-day intervals. All other parameters were statistically processed by the two-way ANOVA on the basis of CRD with a factorial experiment in three replications. The factors included two biochar levels without or with copper (0% biochar (control), 0% biochar with copper, soil amended with 5% walnut shell biochar, 5% walnut shell biochar, 5% walnut shell biochar with copper, 5% methanol-activated walnut shell biochar, 5% methanol-activated walnut shell biochar with copper) and two tetracycline levels (0 and 400 mg/kg soil), which were measured at 10-, 30- and 50-day intervals. One-way and two-way ANOVAs were performed using the SAS software. All parameters were verified for normality using the Anderson–Darling test. Bartlett's test for the homogeneity of variances was used to test the equality of variances in all samples. The means were compared using the Tukey's multiple comparison test for different groups. The correlations between the TC concentration and the other parameters were analyzed by the Pearson correlation coefficient.

Results and discussion

Characterization of walnut shell biochar after activation

High pH and high carbon content of WBC (Table 1) could be related to high pyrolysis temperature. Similar to these results, Ahmed et al. (2012) reported that high pyrolysis temperature can increase the pH of BC because the alkaline elements release from the raw material. They also showed that high temperature can increase carbonization which results in high carbon content in BC. Methanol activation had no effect on the pH of WBC which may be the result of the neutral pH of methanol. However, in the study of Luo et al. (2018) alkaline KOH solution increased the pH of BC. The activation process was also associated with changes in elemental composition and an increase in carbon content. Similar results have been observed in previous studies (Jing et al. 2014; Wang et al. 2020). Figure 2 presents the infrared spectral regions of the WBC (a) and WBCM (b). As illustrated in Fig. 1a, the functional groups of the WBC included the stretching vibration of the OH (lignin and cellulose) at 3436 cm^{-1}, the aliphatic C–H (lignin, cellulose and hemicellulose) at 2908 cm^{-1}, the aromatic C=O at 1703 cm^{-1}, the aromatic C=C stretching at 1566 and 1423 cm^{-1}, stretching vibration of C–O in phenol at 1166, 1119 and 1061 cm^{-1} and aromatic C–H at 871 and 669 cm^{-1}. These findings indicate that the high temperature of pyrolysis with the decomposition of cellulose and lignin can lead to a decrease in polar functional groups and an increase in aromatic structures (Uchimiya et al. 2011; Ahmad 2012). The importance of BC aromatic structures has been proved in the reduction of organic pollutants concentration in the solution medium (Ahmad et al. 2014). After the activation, some changes

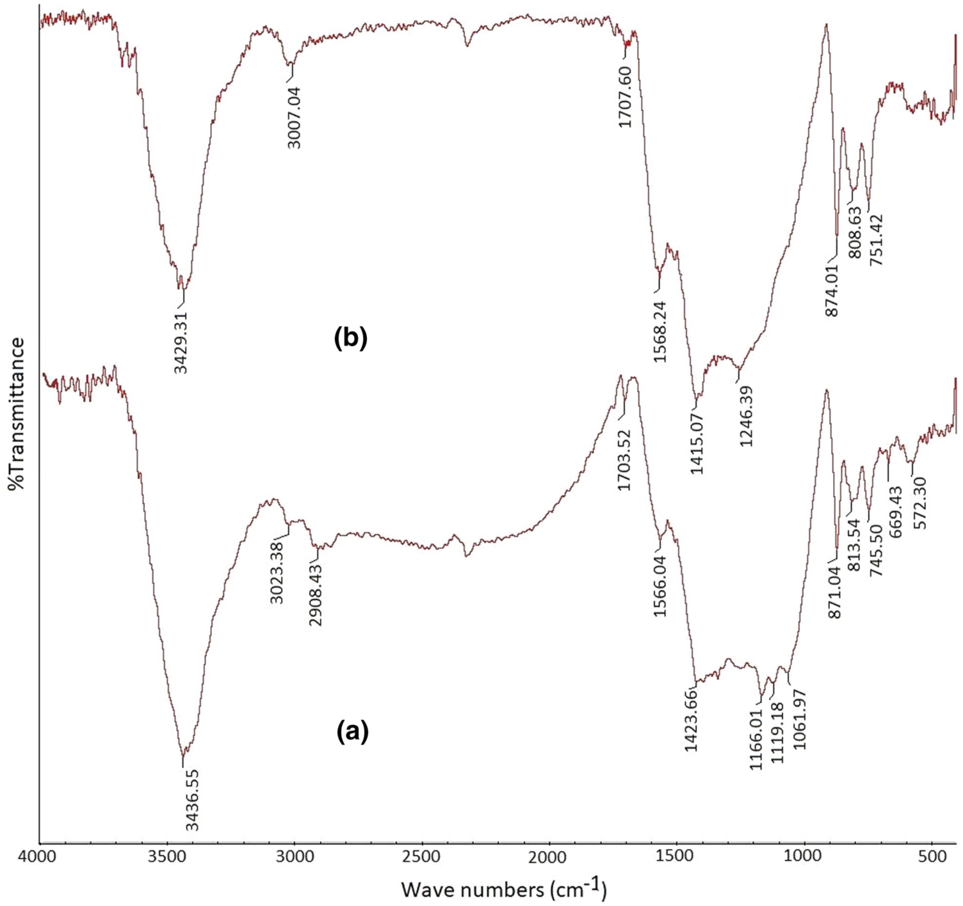

Fig. 2 FTIR spectra of the walnut shell biochar before (**a**) and after **b** activation with methanol

emerged in the WBC functional groups (Fig. 2b). The intensity of the 3436 cm^{-1} peak decreased and two peaks at 1166 and 1061 cm^{-1} were omitted. Also the peak of 1246 cm^{-1} appeared in the figure. The changes of functional groups on the surface of WBC show that the concentration of carbonyl groups decreased after activation process. It seems that the hydroxyl groups were also replaced by -O- alkyl which increased ester groups. These changes indicate that a reaction between the WBC functional groups and methanol occurred during the activation process. Jing et al. (2014) also reported similar results in BC after methanol activation. They showed that the changes in the BC functional groups increased the BC efficiency in decreasing the concentration of TC in the solution medium. Therefore, it is possible that amended soil with WBC and WBCM could affect the environmental behavior of TC as an organic pollutant.

Effect of walnut shell biochar, methanol-activated walnut shell biochar and copper on residual tetracycline concentration in soil

Analysis of variance (ANOVA) of the effect of different treatments on the concentration of tetracycline is given in Table S1 (Supplementary data). The effects of different treatments on the residual concentration of TC during the incubation time are illustrated in Fig. 3. In unamended soil with BC (K = control), the residual concentration of TC decreases, respectively, by 17.9%, 32.5% and 45.2% as compared to the initial concentration (400 mg/kg TC) at 10, 30 and 50 days. In the soil environment, the residual concentration of TC can decrease because of the impact of a wide range of processes such as biodegradation, transformation, sorption and other processes. Hence, it has not been possible to identify the accurate contribution of each process to the reduction of TC residual concentration (Chen et al. 2018a, b). Studies have provided (Rabølle and Spliid 2000; Pan and Chu 2016) information on the positive correlation between the clay content of soil and the decrease of TC residual concentration as a result of the sorption mechanism. However, soil pH affects the speciation of ionizable tetracycline. At alkaline pH (above 7), TC has more negative charges. The electrostatic repulsion between TC and clay surfaces would elevate TC concentration in the solution phase (Wang et al. 2008). Therefore, the accessibility of soil microorganisms to TC has increased (Yue et al 2019) and they can use TC as a substrate. In this study, soil pH was alkaline (Table 2), so in the initial stages the role of sorption were not significant in the decrease in TC residual concentration. Alternatively,

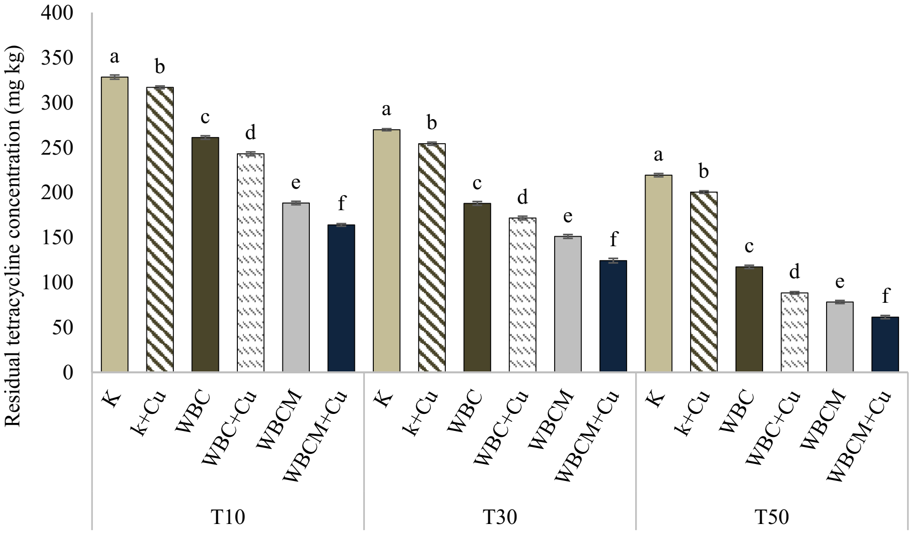

Fig. 3 Residual tetracycline concentration in amended soils with/without the presence of copper. Treatments were: K (control), K + Cu (control with Cu), WBC (biochar), WBC + Cu (biochar with Cu), WBCM (methanol-activated biochar), WBCM + Cu (methanol-activated biochar with Cu), which were measured after 10 (T10), 30 (T30) and 50 (T50) days. The error bars present standard error (mean ± SD, $n = 3$). Different letters represent significant differences between the treatments at each time ($P < 0.05$). Tetracycline concentration was statistically processed by the one-way analysis of variance as a completely randomized design

the increased bioavailability of TC as affected by soil pH can facilitate the reduction of TC residual concentration through biodegradation during the 50-day incubation.

Application of BC significantly ($P < 0.05$) reduced the residual concentration of TC in comparison with unamended soil (Fig. 3). The residual concentrations of TC in WBC-treated soil decreased, respectively, by 20.5%, 30.4% and 40.5% after 10, 30 and 50 days as compared to unamended soil. Previous studies have shown the positive effects of BC on the TC residual concentration reduction through sorption (Liu et al. 2020) and the changes of soil properties (Yue et al 2019). Both direct and indirect effects of biochar have a significant role in the reduction of TC residual concentration and soil health improvement as well (Li et al. 2019). It is well known that these effects of BC as an organic matter mostly depend on BC properties. Li et al.)2016(stated that the enhancement of organic matter content in soil affects the extractable TC fractions (soluble and desorbable fractions) through the formation of stable complexes. As a result, both the extractability of TC and the amount of residual concentration of TC are reduced. Although the exact mechanism of reaction between BC and TC in the soil is not clear, it seems that because of having aromatic structures (Fig. 2a) its application as an organic material can form stable complexes with TC which leads to the reduction of residual concentration of TC in the early stages by reduction of the extractable fractions of TC. Also, it has been reported that the porous structure of the BC has affected the decrease of TC residual concentration through the diffusion process over time (Ngigia et al. 2020.

In WBCM-treated soils, the residual concentration of TC was significantly decreased by 42.6%, 46.5% and 64.3%, respectively, as compared to unamended soil after 10, 30 and 50 days (Fig. 3, $P < 0.05$). Also, in WBCM-treated soils, the residual concentration of TC was significantly decreased by 27.9%, 19.5% and 33.3%, respectively, as compared to WBC-treated soil after 10, 30 and 50 days (Fig. 3, $P < 0.05$). Previous studies have provided valuable information on the effects of BC activation in order to enhance its efficiency in the reduction of TC concentration in the solution (Wang et al. 2020). Jing et al. (2014) showed that methanol-activated BC through the process of sorption could efficiently decrease TC concentration up to 100% in the solution medium. In the terrestrial environments, the complexity of the system affects the BC influence on the reduction of TC concentration (Yue et al 2019). Therefore, it seems that the effects of BC in decreasing TC residual concentration in WBCM-treated soils could be related to the formation of stable complexes, the reduction of TC extractability in the early stages as well as biodegradation through time. In general, the results of this research indicated that both WBC and WBCM application in soil accelerate the decrease in TC residual concentration because of the decrease in extractable fractions in the early stages.

Regardless of the application and the type of the BC, the presence of Cu in the soil led to a decrease in the residual concentration of TC)Fig. 3). In WBC + Cu treatments, the presence of Cu decreased the residual concentration of TC by 6.9%, 8/6% and 24.7%, respectively, in comparison with WBC treatments after 10, 30 and 50 days ($P < 0.05$). Also, in WBCM + Cu, the residual concentration of TC was significantly decreased by 12.9%, 17.8% and 21.5% as compared to WBCM after 10, 30 and 50 days (Fig. 3, $P < 0.05$). Although the mechanism of interaction between TC and metals is not clearly identified in the soil environment, studies in aquatic systems have helped us to better understand the simultaneous environmental behavior of these pollutants in soil systems. The effects of TC and Cu on their mutual chemical behavior could probably be influenced by the soil solution pH. At alkaline pH, the hydroxyl group of TC have the negative charge (Wang et al. 2008). In this condition, TC can easily form complexes with cationic ions such as copper, magnesium, cadmium and lead in the soil system. Among these metals, Cu could form strong complexes with TC (Sassman and Lee 2005; Zhao et al. 2013). Similar to the BC effects, the stability of TC–Cu complexes could probably affect TC extractability and could reduce the residual concentration of TC in the initial stages. Cu can also facilitate the decrease of TC residual concentration through the "cation bridge" mechanism (Jia et al. 2013). In addition, studies have reported (Yue et al, 2019) that the formation of TC complexes with metals can lead to the presence of TC in the soil solution phase. In this case, the accessibility of microorganisms to TC increases over time and contributes to biodegradation. However, the accessibility of microorganisms to TC in TC–Cu complexes is lower than free TC. Therefore, the contribution of biodegradation to the reduction of TC concentration decreased. It seems that the presence of copper has a positive effect on reducing the residual concentration of TC because of reduction the extraction capacity of TC and biodegradation.

Effect of tetracycline on copper concentration in soil

Analysis of variance (ANOVA) of the effect of different treatments on the concentration of copper is given in Table S2 (Supplementary data). In the present study, due to the importance of the interaction between copper and TC in the medium, changes in Cu-DTPA concentration in the presence of TC were also investigated (Fig. 4). The results showed that the presence of TC in both amended and unamended soils increased Cu-DTPA concentration. In unamended soils with pollution (TC400), as compared with unpolluted soils (TC0), the maximum increase in Cu-DTPA concentration was 72.8% (Fig. 4, $P < 0.05$) after 30 days. The increase in Cu-DTPA concentration may be related to the alkaline pH of the soil. Previous studies (Wang et al. 2008) have shown that the TC–Cu complex at alkaline pH is mostly neutral, which has less ability than Cu^{2+} to form stable complexes with exchange surfaces in soil. In amended soils with pollution (TC400), as compared with unamended soils without pollution (K + Cu, TC0), maximum increase in Cu-DTPA concentration (110.3%) was found in WBCM treatment after 50 days (Fig. 4, $P < 0.05$). In these treatments, the concentration of Cu-DTPA has probably increased because of the presence of TC and biochar properties. Uchimiya et al. (2011) showed that BC produced at a high pyrolysis temperature has no effect on the reduction of heavy metals concentration, especially Cu, because of development of aromatic structures and the reduction of oxygen-containing functional groups. However, decreasing trend of Cu-DTPA concentration due to the usage of BC may be attributed to the application of organic matter which enhances soil

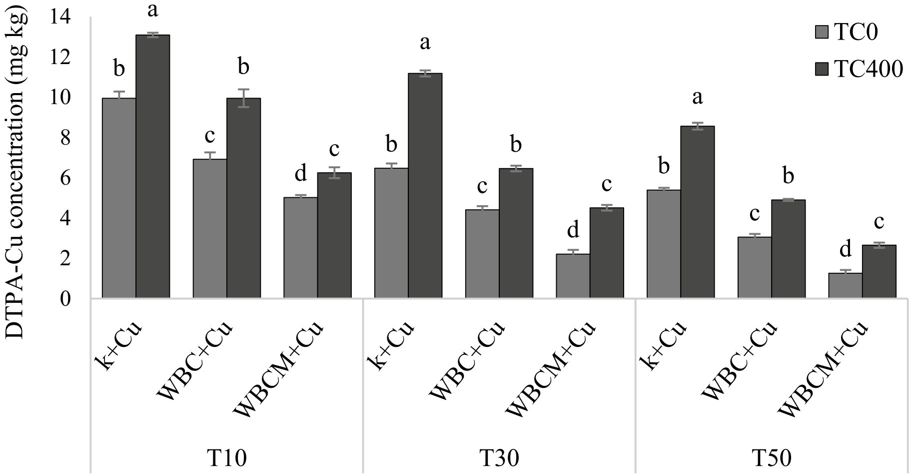

Fig. 4 DTPA-Cu concentration in amended soils and the presence of tetracycline. Treatments were: K + Cu (control with Cu), WBC + Cu (biochar with Cu), WBCM + Cu (methanol-activated biochar with Cu), TC0 (unpolluted soil), TC400 (polluted soil with tetracycline), which were measured after 10 (T10), 30 (T30) and 50 (T50) days. The error bars present standard error (mean ± SD, $n = 3$). Different letters represent significant differences between the treatments at each time ($P < 0.05$). Coppre concentration was statistically processed by the two-way analysis of variance as a completely randomized design with factorial experiment

negative charge. In addition, TC and Cu-DTPA concentration (Table 3) showed a weak positive correlation ($P < 0.005$, $r = 0.247$). Therefore, although the presence of TC in the soil increased the Cu-DTPA concentration, the positive effect of Cu on reducing the TC concentration was more significant in this study. In general, this study showed that the chemical interaction between Cu and TC in the soil environment affects the chemical behaviors of each. Hence, it is unavoidable to consider the contribution of their interactions in soil management and improving soil health conditions.

Effect of walnut shell biochar and methanol-activated walnut shell biochar on biological effects of tetracycline in soil

Analysis of variance (ANOVA) of the effect of different treatments on microbial biomass, dehydrogenase and urease activities is given in Tables S2 and S3 (Supplementary data). In this study, the changes of biological effects of TC as affected by WBC and WBCM were investigated on microbial biomass, dehydrogenase and urease activities. The levels of Cu have no significant differences on soil biological parameters ($P > 0.05$, the data were not shown). The effect of different treatments on microbial biomass is shown in Fig. 5. In accordance with previous studies (Thiele-Bruhn and Beck 2005), TC-polluted soil caused a significant decrease ($P < 0.05$) in microbial biomass after 10 and 30 days. WBC and WBCM application reduced the negative effects of TC on soil microbial biomass. In WBC and WBCM treatments, TC decreased microbial biomass by 14.7% and 16.5%, respectively ($P < 0.05$) at 10 days. Comparison between amended and unamended treatments (Fig. 5) showed that WBC and WBCM had no significant effect on microbial biomass in unpolluted during the incubation time. The available nutrients in WBC and WBCM probably decreased as a result of the high temperature of pyrolysis (Luo et al. 2013; Zhang

Table 3 Pearson correlation coefficients between tetracycline concentration and other parameters

	Tetracycline concentration	Cu-DTPA concentration	Microbial biomass	Urease activity	Dehydrogenase activity
Tetracycline concentration	1.000				
Cu-DTPA Concentration	0.248*	1.000			
Microbial Biomass	−0.769**	−0.297*	1.000		
Urease Activity	−0.863**	−0.253*	0.682**	1.000	
Dehydrogenase Activity	−0.297*	−0.029 ^{ns}	0.354**	0.322**	1.000

**Highly significant ($P < 0.001$), *significant ($P < 0.005$), ns: no significant ($P > 0.005$)

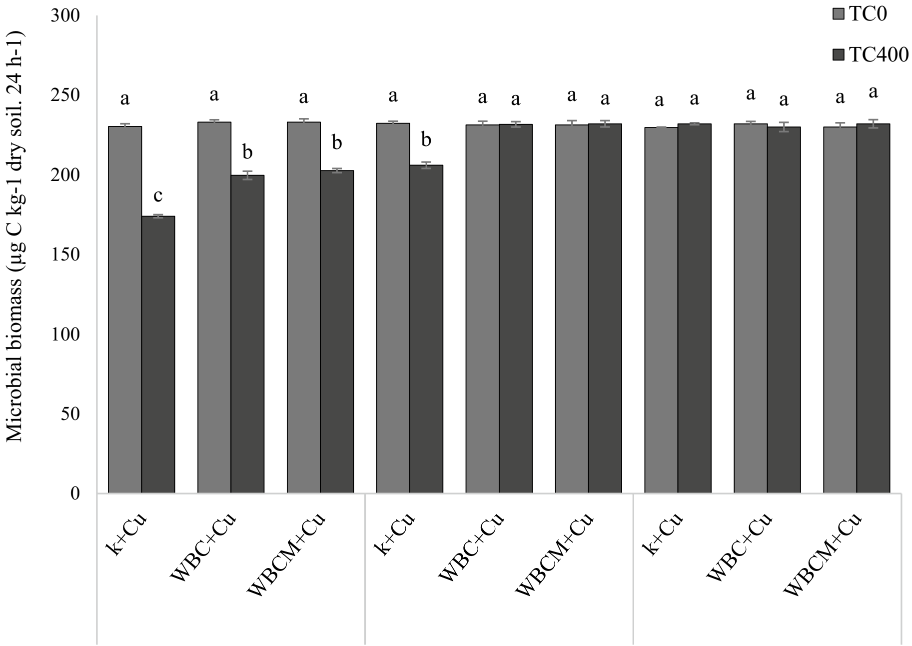

Fig. 5 Rates of microbial biomass in soil samples. Treatments were: K + Cu (control with Cu), WBC + Cu (biochar with Cu), WBCM + Cu (methanol-activated biochar with Cu), TC0 (unpolluted soil), TC400 (polluted soil with tetracycline), which were measured after 10 (T10), 30 (T30) and 50 (T50) days. The error bars present standard error (mean ± SD, $n = 3$). Different letters represent significant differences between the treatments at each time ($P < 0.05$). Microbial biomass was statistically processed by the two-way analysis of variance as a completely randomized design with factorial experiment

et al 2014), so they had no effect on microbial biomass during the experiment.

However, correlation analysis between TC concentration and microbial biomass (Table 3) showed a high negative correlation ($P < 0.001$, $r = -0.769$) between decrease of TC residual concentration and improvement of microbial biomass. Studies have shown that (Hammesfahr et al. 2011; Liu et al. 2012) the decrease of TC extractable fractions could potentially affect the bioavailability of antibiotics to microorganisms. Therefore, it seems that WBC and WBCM have reduced the accessibility of microorganisms to TC in the early stages through complex formation with TC. As a result, TC was less used as a substrate by microorganisms and its effects on soil microbial biomass were reduced. In addition, even if the biochar produced at a high temperature cannot provide nutrients for the growth of microorganisms, studies have shown that BC can provide a niche for antibiotic-tolerant bacterial strains (Wang et al. 2017). This can contribute to the biodegradation of TC and improvement of soil microbial activities over time. However, the selective effects of TC can not be well indicated in microbial biomass (Thiele-Bruhn and Beck 2005). Therefore, this indicator is not the only one to determine soil health.

Changes of urease and dehydrogenase activity during the incubation are shown in Fig. 6. Urease and dehydrogenase enzymes responded differently to TC soil pollution

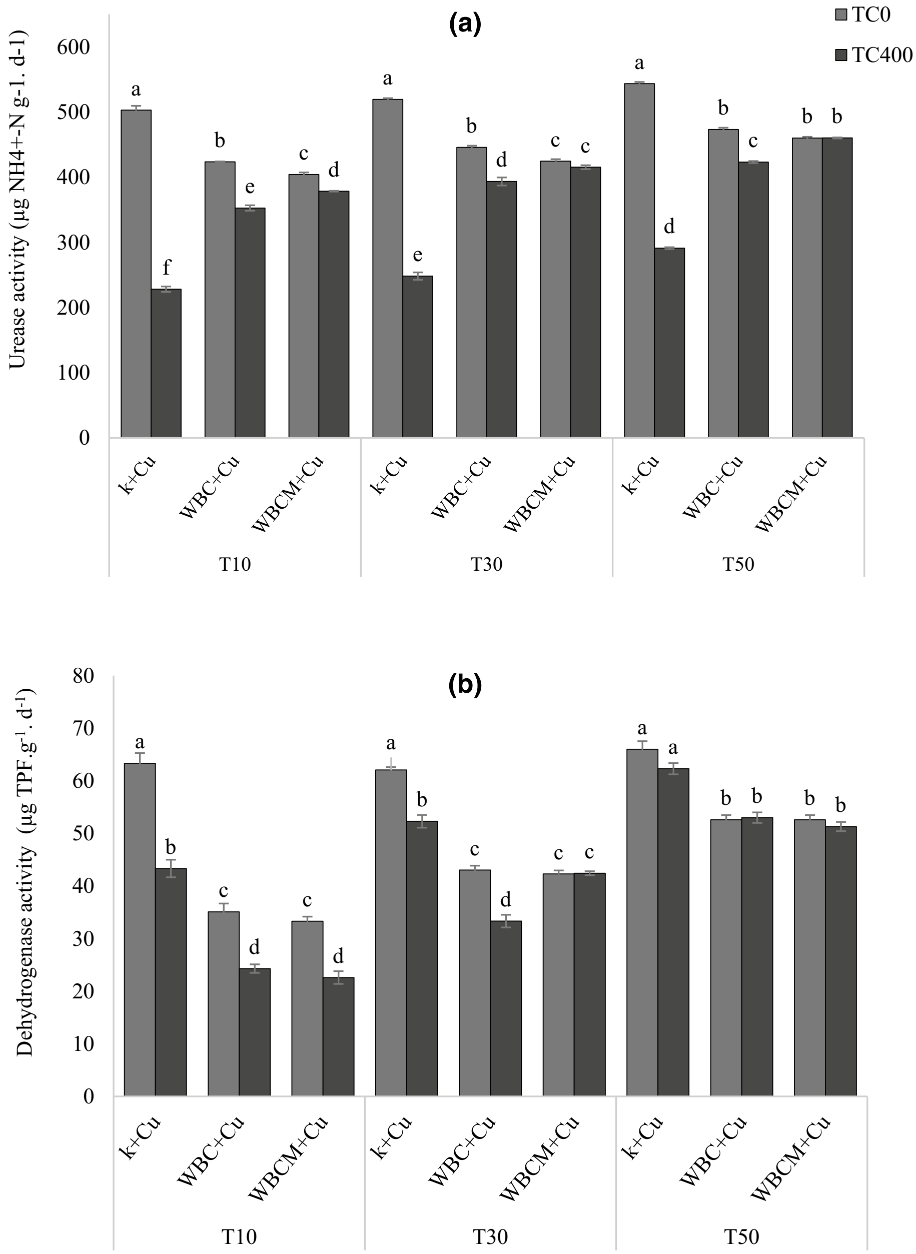

Fig. 6 The amount of **a** urease and **b** dehydrogenase activities. Treatments were: K + Cu (control with Cu), WBC + Cu (biochar with Cu), WBCM + Cu (methanol-activated biochar with Cu), TC0 (unpolluted soil), TC400 (polluted soil with tetracycline), which were measured after 10 (T10), 30 (T30) and 50 (T50) days. The error bars present standard error (mean ± SD, $n = 3$). Different letters represent significant differences between the treatments at each time ($P < 0.05$). Urease and dehydrogenase activities were statistically processed by the two-way analysis of variance as a completely randomized design with factorial experiment

and the application of WBC and WBCM. The lowest urease enzyme activity (228.2 µg NH_4^+-N g^{-1}. d^{-1}) was measured in unamended TC-polluted soil after 10 days. In unamended soil, the inhibitory effects of TC on urease activity were still significant after 50-day incubation (Fig. 6a, $P > 0.05$). The application of WBC and WBCM in unpolluted soil decreased urease activity. However, their application in TC-polluted soil weakened the negative effects of TC on urease activity. In amended soils, the maximum decrease in urease activity (19.6%) was found in WBCM treatments without pollution (TC0) after 10 days (Fig. 6a, $P > 0.05$). During the incubation time, WBC reduced the negative effects of TC, and WBCM caused these negative effects on urease activity to be significant (Fig. 6a, $P > 0.05$) at 10 days. Also, the correlation analysis between TC concentration and urease activity (Table 3) showed the highest negative correlation between these two parameters ($P < 0.001$, $r = -0.862$). The negative effects of tetracycline on urease activity have been shown in previous studies (Liu et al. 2015; Molaei et al. 2017; Liang et al. 2020). TC can reduce urease activity even at low concentrations (Wei et al. 2009). Molaei et al. (2017) also stated that the toxicity of TCs on urease activity is higher than other groups of antibiotics. Therefore, even little changes of TC concentration can affect the activity of the urease. Although the application of BC may reduce urease activity due to the sorption and stabilization of extracellular enzymes (Huang et al. 2017), studies have reported that BC can act as a barrier to prevent the negative effect of pollutants on enzyme activities through the stabilization of organic pollutants as well as enzymes.

In contrast, dehydrogenase activity showed different responses to TC and soil amendment application (Fig. 6b). In unamended treatments, dehydrogenase activity was significantly reduced in TC-polluted soil by 31.5% and 15.7% after 10 and 30 days, respectively. Studies have reported different results regarding the effect of TC on dehydrogenase activity. Thiele-Bruhn and Beck (2005) observed that TC did not decrease dehydrogenase activity, while Liu et al. (2015) reported negative effects of TC on the decrease of dehydrogenase activity even at high concentrations. Liu et al. (2009) stated that different responses of dehydrogenase activity to TC could be related to different determinative factors affecting dehydrogenase activity. Therefore, changes in dehydrogenase activity during the experiment may have not been affected only by TC. The application of WBC and WBCM in unpolluted soil significantly decreased dehydrogenase activity (Fig. 6b, $P < 0.05$). In amended soils, the maximum decrease in dehydrogenase activity (47.4%) was found in WBCM treatments without pollution (TC0) after 10 days. Similar to the study of Liang et al. (2020), WBC and WBCM probably reduced dehydrogenase activity due to high level of application (5%w/w). The high content of BC can have high concentration of organic compounds such as phenols which are toxic to microorganisms and enzymes. Also, microorganisms and enzymes can be absorbed by the micropores and mesopores of BC. In this case, BC can inhibit microbial growth and enzymatic activities (Liang et al. 2020). However, the presence of the WBC and WBCM in TC-polluted soil over time could help to reduce the negative effects of TC on dehydrogenase activity. Also, correlation analysis (Table 3) showed a weak negative correlation ($P < 0.005$, $r = -0.297$) between the decrease in concentration of TC and improvement in dehydrogenase activity. Therefore, although the exact effect of TC and the application of WBC and WBCM on dehydrogenase activity is not clear, it seems that soil remediation with WBC and WBCM has a positive effect on dehydrogenase activity improvement in the long term. In general, the study of biological indicators during the incubation time showed that amended TC-polluted soil with WBC and WBCM could possibly affect the bioavailability of TC through the formation of stable complexes with TC which can help to ameliorate soil biological activities (microbial biomass, urease and dehydrogenase activities). Improvement in biological indicators is a sign of soil health improvement. Therefore, it seems that amendment of TC-polluted soils with WBC and WBCM can be an effective measure to improve soil health. However, further studies are required to accurately determine the effects of the BC on the chemical and biological behavior of TC as well as its fate on soil.

Conclusion

The results of the present study showed that the application of WBC and WBCM as an amendment significantly improved the reduction of TC residual concentration in the soil environment. The development of aromatic structure on WBC and changes of functional groups on WBCM have led to the formation of stable complexes with TC. These complexes affect the extractable fractions of TC and reduce the residual concentration of TC in the soil at early stages. Similar to aquatic systems, the presence of Cu in soil had the same effects on the chemical behavior of TC. There is still little information on the effective mechanisms of interactions between metal and TC in the soil. Similar to the effect of BC, it seems that Cu has a positive effect on the reduction of TC extractability through the formation of stable complexes. The study of Cu-DTPA concentration showed that the application of BC and the presence of TC have a negative effect on reducing the concentration of Cu-DTPA in the soil environment. The development of aromatic structures has had a negative effect on the efficiency of BC to form the complexes with Cu. TC has also reduced the capacity of Cu to form complexes with exchange sites by the formation of a stable complex with Cu. The application of WBC and

WBCM in TC-polluted soil weakened the negative effects of TC on biological indicators, especially microbial biomass and urease activity. In addition, the application of BC can contribute to the biodegradation of tetracycline over time through the increasing niche for tolerant bacterial strains. The results of this study showed that the application of BC in TC-polluted soil can help to improve soil health.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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